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Purification and spectroscopic characterization of a recombinant chloroplastic hemoglobin from the green unicellular alga Chlamydomonas eugametos.

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Hemoglobins (Hb), which have the important task of delivering molecular oxygen by facilitating its reversible binding to the heme, are now thought to have evolved in all groups of organisms including prokaryotes, fungi, plants and animals. Our recent finding of a light-inducible chloroplastic Hb in the green unicellular alga Chlamydomonas eugametos has further extend this idea, while raising questions about the function that an Hb could play in a high oxygen environment such as in the chloroplast. In order to understand the role played by this new Hb, we have undertaken its biochemical characterization. To facilitate the characterization of Chlamydomonas Hb, which represents less than 0.01% of the soluble protein in the green alga, the protein has been expressed in Escherichia coli and purified to apparent homogeneity. The purified recombinant protein possesses a non-covalently bound iron-protoporphyrin IX heme. The oxy form of the recombinant Hb. purified directly from bacterial cells, is very stable, with a measured half-life of 7 days at pH 8 and has an ultraviolet/visible spectrum similar to those of the related cytoplasmic Hbs of the ciliated protozoa Paramecium and Tetrahymena and of the cyanobacterium Nostoc commune. In contrast to what has been reported for oxymyoglobins and oxyhemoglobins, the dioxygen molecule bound to the L1637 Hb can be reduced by the electron-transfer mediator phenazine methosulfate in the presence of NADPH, indicating that the heme pocket of Chlamydomonas Hb may be more accessible to small molecules. With regard to this we found that when the small reducing agent sodium dithionite is used to reduce the met form, it must be removed anaerobically from the Hb prior to oxygenation of the protein to stably produce the oxy form. Otherwise, the oxy form is obtained readily from the met form under an oxygenic atmosphere when ferredoxin and ferredoxin NADP+ reductase are used to enzymically reduce the Hb. Finally, the spectra of the deoxy and met forms were unusual, the heme being partly low-spin at physiological pH. These results confirm the existence of a reversible oxygen-binding protein in the chloroplast of C. eugametos. The unusual spectral and biochemical properties of the protein may reflect a specialized

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The metabolic effects of native and transgenic hemoglobins on plants.

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The strictly aerobic bacterium Vitreoscilla expresses a hemoglobin-like protein, VHb, when subjected to oxygen stress. When expressed in plants, this has several intriguing physiological effects, such as improving the overall growth rate, speeding germination and flowering, and increasing the productivity of certain oxygen-requiring metabolic pathways. Although the mechanisms behind the effects of VHb in heterologous hosts are not yet fully characterized, it has been suggested that VHb facilitates oxygen transport and/or storage. This hypothesis is supported by the kinetic properties of VHb, which allow very rapid dissociation of oxygen from the protein.

Publication Types:
- Review
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Rice hemoglobins. Gene cloning, analysis, and O2-binding kinetics of a recombinant protein synthesized in Escherichia coli.

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Although nonsymbiotic hemoglobins (Hbs) are found in different tissues of dicots and monocots, very little is known about hb genes in monocots and the function of Hbs in nonsymbiotic tissues. We report the cloning and analysis of two rice (Oryza sativa L.) hb genes, hb1 and hb2, that code for plant Hbs. Rice hb1 and hb2 genes contain four exons and three introns, as with all of the known plant hb genes. At least three copies of the hb gene were detected in rice DNA, and analysis of gene expression shows that hb1 and hb2 are expressed in leaves but only hb1 is expressed in roots. A cDNA for rice Hb1 was expressed in Escherichia coli, and the recombinant Hb (rHb1) shows an unusually high affinity for O2 because of a very low dissociation constant. The absorbance spectra of the ferric and deoxyferrous rHb1 indicate that, in contrast to symbiotic Hbs, a distal ligand is coordinated to the ligand-binding site. Mutation of the distal His demonstrates that this residue coordinates the heme Fe of ferric and deoxyferrous rHb1 and stabilizes O2 in oxy-rHb1. The biochemical properties of rice rHb1 suggest that this protein probably does not function to facilitate the diffusion of O2.

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1: Plant Physiol 1997 Jan;113(1):45-57 Related Articles, Nucleotide, Books, LinkOut

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Cell-specific expression of the promoters of two nonlegume hemoglobin genes in a transgenic legume, Lotus corniculatus.

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The promoters of the hemoglobin genes from the nitrogen-fixing tree Parasponia andersonii and the related nonnitrogen-fixing Trema tomentosa both confer beta-glucuronidase reporter gene expression to the central zone of the nodules of a transgenic legume, Lotus corniculatus. beta-Glucuronidase expression was high in the uninfected interstitial cells and parenchyma of the surrounding boundary layer and was low in the Rhizobium-infected cells. This contrasts with the expression of both the P. andersonii hemoglobin protein in P. andersonii nodules and the endogenous Lotus leghemoglobins that are expressed in the infected cells at very high levels. The expression pattern of the P. andersonii and T. tomentosa hemoglobin promoters in L. corniculatus resembles that of a nonsymbiotic hemoglobin gene from Casuarina glauca, which was introduced into this legume, and suggests that only the nonsymbiotic functions of the P. andersonii promoter are being recognized. Deletion of the distal segments of both the P. andersonii and T. tomentosa promoters identified regions important for the control of their tissue-specific and temporal activity in Lotus. Potential regulatory elements, which enhance nodule expression and suppress nonnodule expression, were also identified and localized to a distal promoter segment. A proximal AAGAG motif is present in the P. andersonii, T. tomentosa, and nonsymbiotic Casuarina hemoglobin genes. Mutation of this motif in the P. andersonii promoter resulted in a significant reduction in both the nodule and root expression levels in L. corniculatus. Some of the regulatory motifs characterized are similar to, but different from, the nodulin motifs of the leghemoglobins.

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1	15	nonsymbiotic adj hemoglobin or haemoglobin near9 (plant vigor or agronomic)	USPAT; US-PGPUB; DERWENT	2002/08/13 13:18
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